RESEARCH

Deficiency in Induction but Not Expression of LTP in Hippocampal Slices from Young Rats

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Abstract

In this study we examine developmental changes between postnatal day (PND) 4 and 14 in synaptic transmission and plasticity in the CA1 region of hippocampal slices. We confirm previous results that tetanus-induced long-term potentiation (LTP) in field recordings is diminished in slices from younger animals. LTP in whole-cell current-clamp recordings is also diminished in younger animals. However, robust LTP can be induced in young animals if sufficient postsynaptic depolarization is provided during LTP induction. Furthermore, we find differences in synaptic transmission between PND 4 and 14, suggesting that the depolarization during tetanic stimulation in young tissue is ineffective to produce LTP. These results indicate that the smaller potentiation in field recordings in slices from younger animals is attributable to insufficient postsynaptic depolarization during LTP induction rather than a defect in expression mechanisms.

Introduction

Long-term potentiation (LTP), as characterized in the CA1 region of the hippocampus, is thought to have a role in learning and memory (Bliss and Collingridge 1993; Bannerman et al. 1995; Barnes 1995). Similar cellular mechanisms may also have critical roles during the development of the central nervous system (Cline et al.

1987; Constantine-Paton et al. 1990). Both types of neuronal plasticity are activity-dependent (Archer et al. 1982; Constantine-Paton et al. 1990; Bliss and Collingridge 1993); both are thought to require Hebbian association of pre- and postsynaptic activity (Malinow and Miller 1986; Collingridge and Bliss 1987; Gustafsson et al. 1987; Fregnac et al. 1988; Greuel et al. 1988) and the activation of *N*-methyl-D-aspartate (NMDA) receptors (Cline et al. 1987; Collingridge and Bliss 1987). Recently, LTP was linked to critical period plasticity in the primary somatosensory cortex (Crair and Malenka 1995) and the primary visual cortex (Kirkwood et al. 1995). In these two studies, robust LTP was seen early in neocortical development. LTP in the hippocampus, however, may behave differently. Previous reports have indicated that hippocampal LTP is either difficult to generate (Harris and Teyler 1984; Muller et al. 1989) or not possible (Dudek and Bear 1993; Bolshakov and Siegelbaum 1995) in rats <8 days old.

It is not clear why there is less LTP in the hippocampus in younger animals. In general terms, one or more of the steps involved in the induction, expression, or maintenance of LTP may be defective (see Discussion). For example, sufficient depolarization during induction is necessary to generate LTP, and this may be more difficult to achieve in young animals. Alternatively, the calcium/calmodulin-dependent protein kinase II (CaMKII), an enzyme important to generate LTP, shows a developmentally controlled expression pattern that begins during the first postnatal week (Kelly et al. 1987; Scholz et al. 1988). A third possibility is that synaptic potentiation is already maximally saturated in the hippocampus of young rats (Bolshakov and Siegelbaum 1995).

Excitatory synapses in CA1 hippocampus, as in most of the regions studied in the vertebrate

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central nervous system, transmit through the actions of glutamate on AMPA (α -amino-3-hydroxy-5-methyl-4-isoxazolepropionate) and NMDA-type glutamate receptors (Collingridge et al. 1983; Collingridge and Lester 1989). On binding transmitter, these receptors have markedly different biophysical properties. AMPA receptors can conduct at hyperpolarized potentials, whereas NMDA receptors require depolarized potentials (Mayer et al. 1984). These receptors also have different roles in plasticity. Transient NMDA receptor activity is required to trigger LTP, whereas AMPA receptors are used in the expression of LTP (Collingridge and Bliss 1987). Although both receptor types are expressed on individual neurons (Petralia and Wenthold 1992; Good et al. 1993; Eshhar et al. 1993; Martin et al. 1993; Hollmann and Heinemann 1994; Petralia et al. 1994; Siegel et al. 1994, 1995), the distribution of receptors on individual synapses is less clear. Recently, we and others provided electrophysiological evidence for the existence of synapses that transmit with only NMDA receptors (Kullman 1994; Isaac et al. 1995; Liao et al. 1995; Durand et al. 1996). In our previous study we estimated that ~60% of synapses were of this type, with the remainder containing both functional AMPA receptors and NMDA receptors. The fraction of synapses with only NMDA receptors may be important to the induction of LTP, as synaptic regions may fail to depolarize sufficiently during tetanic stimulation if they contain no AMPA type receptors.

In this study, we have investigated developmental changes of LTP in rat hippocampal CA1 region as measured with extracellular field, whole-cell voltage-clamp and whole-cell current-clamp recordings. Consistent with previous studies, LTP is diminished in the early stages of development as measured with field and current-clamp recordings. However, robust LTP was generated when postsynaptic depolarization was provided directly during LTP induction in whole-cell voltage-clamp recordings. This suggests that the small LTP in young rats is attributable to insufficient postsynaptic depolarization during the induction protocol. In support of this, we find that synaptic transmission in baseline conditions and during tetanic stimulation produces different depolarization at different ages. The results indicate that the expression mechanisms for LTP and the biochemical components required for LTP induction downstream of NMDA receptor activation exist at the ages examined (postnatal day 5 to 14).

Materials and Methods

BRAIN SLICE PREPARATION AND RECORDING

Hippocampal slices (400 μ m) were prepared with a chopper or a vibratome from 4- to 14-day-old rats as described previously (Malinow and Tsien 1990). The slices were submerged and superfused with a bath solution containing 119 mM NaCl, 2.5 mM KCl, 2.5 mM CaCl_2 , 1.3 mM MgCl_2 , 26.2 mM NaHCO_3 , 1 mM NaH_2PO_4 , 11 mM glucose, and gassed with 95% O_2 and 5% CO_2 . Picrotoxin (100 μ M) was added to block GABA_A (γ -aminobutyric acid) inhibitory postsynaptic currents. CA3 and CA1 regions were separated surgically to prevent epileptiform activity. Synaptic transmission was elicited by passing a brief (<200 μ sec) current pulse through an extracellular glass-stimulating electrode (filled with 2 M NaCl) placed in CA1 stratum radiatum ~50 μ m from *S. pyramidale*.

Whole-cell recordings were obtained under visual guidance from cells two to three layers below the surface with a patch electrode (2–4 M Ω tip resistance) at a temperature of 24–28°C. Series resistance was monitored and remained constant during an experiment (5–30 M Ω). The internal solution contained 100 mM CsMeSO₃, 40 mM HEPES, 2 mM NaATP, 0.3 mM GTP, 5 mM MgCl_2 , 5 mM glutathione, and 0.2 mM EGTA (pH 7.2 with CsOH). In some experiments, 100 mM caesium gluconate was used instead of 100 mM CsMeSO₃. No obvious difference was noticed between these two compounds in our whole-cell recordings. In current-clamp experiments, 100 mM potassium gluconate was used. Unless stated otherwise, holding potential was kept constant at a level between –60 mV and –70 mV, and the stimulation frequency was kept at 1/6 Hz or 1/3 Hz. In some experiments (Fig. 7, below), the stimulus level was decreased by small amount (0.05 V) until reaching a level that produced only failures for 75–100 trials after obtaining whole-cell recordings. That stimulus level is defined as a subminimal stimulus. In all voltage-clamp experiments, LTP was induced by pairing 2-Hz presynaptic stimulation for 75 sec with postsynaptic depolarization (–10 mV). In all current-clamp experiments, LTP was induced by a tetanus protocol that consisted of 10 trains (separated from each other by 3 sec) of nine stimuli at 100 Hz.

In field excitatory postsynaptic potential (EPSP) recordings, an extracellular glass recording electrode (filled with the above bath solution) was

placed in CA1 stratum radiatum about 100–200 μm from an extracellular glass-stimulating electrode (filled with 2 M NaCl). Unless stated otherwise, stimulation frequency was kept at 1/30 Hz. LTP was induced by a tetanus protocol that consisted of five trains (separated by 30 sec) of nine stimuli at 100 Hz.

WHOLE-CELL SYNAPTIC AMPLITUDE MEASUREMENT AND DISTRIBUTION ESTIMATE

Individual excitatory postsynaptic current (EPSC) peak amplitudes were determined by averaging the current over a fixed 1- to 4-msec window encompassing the peak amplitude and subtracting the average current during a window prior to the stimulus (see Fig. 5, below). For a given experiment, the same windows were used on recorded sweeps in all experimental conditions. In some experiments, an additional 10-msec window placed 40-msec after the peak was used to estimate NMDA current at a depolarized potential (+55 mV). The same windows placed during a period with no stimulus gave an estimate of the baseline noise. Density estimates of EPSC amplitudes in an epoch were generated by summing all Gaussian kernels with a standard deviation one-half of the noise, each centered about an EPSC amplitude value on the x -axis (amplitude axis) (Silverman 1986; Malinow 1991; Liao et al. 1992). A similar method was used to estimate the noise density distribution. Such a method will overestimate the width of noise density distribution by 10%. Let noise s.d. (standard deviation) = 1, Gaussian kernel s.d. = 0.5; therefore, the noise variance = 1, Gaussian kernel variance = 0.25; therefore, estimated noise distribution variance = $1 + 0.25 = 1.25$, its s.d. = $(\text{variance})^{1/2} = 1.12$.

FRACTION OF POSTSYNAPTICALLY SILENT SYNAPSES

The failure rate was quantified by measuring the amplitude of events and noise and constructing amplitude distribution density estimates (Figs. 1B,C; see Fig. 8, below). The failure peak was located near zero amplitude and matched the distribution of the noise. The proportion of synaptic failures for an epoch of responses was estimated by doubling the fraction of responses with amplitude less than the location of the failure peak. We estimate the fraction of release occurring at pure NMDA synapses by comparing the failure rate at

Figure 1: Tetanus-induced LTP of field EPSP in CA1 hippocampus is smaller in younger rats. (A) A small amount of LTP is induced in a hippocampal slice from a 5-day-old rat (a48s). (Top) Average field EPSP responses before (60 consecutive trials) and after (100 consecutive trials) tetanus. An average of responses obtained in 10 mM CNQX was digitally subtracted from raw traces to generate above averages. (Bottom) Field EPSP amplitude plotted vs. time in the same experiment. Arrow denotes delivery of tetanic protocol (see Materials and Methods). CNQX (10 mM) applied where indicated, blocks responses. (B) Similar plots as A from another experiment with larger LTP in a 13-day-old rat (a52o). (C,D) Potentiation of field EPSP amplitudes at 15 and 60 min post-tetanus plotted vs. animal age. Synaptic potentiation is correlated positively with animal age (C: $n=30$, $R=0.61$, $P<0.001$; D: $n=30$, $R=0.49$, $P<0.01$).

depolarized (F_d) and hyperpolarized (F_h) potentials. We assume that the average probability of release (Pr) at releasing sites containing only NMDA receptors or mixed AMPA and NMDA receptors is similar. This assumption is reasonable given the observation that the NMDA to AMPA ratio in synaptic currents remains constant when the probability of release is modified 16-fold (Perkel and Nicoll 1993). The failure rates at depolarized and hyperpolarized potentials are approximated by $F_h = (1 - Pr)^{n_{AN}}$ and $F_d = (1 - Pr)^{(n_{AN} + n_N)}$, where n_{AN} is the number of releasing sites containing both AMPA and NMDA receptors and n_N is the number of releasing sites containing only NMDA receptors. Rearrangement yields the fraction of pure NMDA synapses: $n_N /$

$(n_{AN} + n_N) = 1 - (\ln F_h / \ln F_d)$. This is the same equation obtained by assuming a low probability of release (Hessler et al. 1993; Rosenmund et al. 1993) as described previously (Liao et al. 1995). This estimate will be affected in part by the recording noise. More noise will lead to counting events (real positive amplitude) as failures. Because the noise is greater at depolarized potentials, this leads to an overestimate of failures at depolarized potentials, minimizing the difference in failures at depolarized and hyperpolarized potentials. There was no systematic increase in noise with age, arguing against this as an artefactual basis for the age-dependent change in pure NMDA transmission.

FIELD EPSP AMPLITUDE MEASUREMENT AND INPUT–OUTPUT CURVE

Individual field EPSP peak amplitudes were determined by averaging the current over a fixed 1- to 4-msec window encompassing the peak amplitude and subtracting a baseline estimate. For a given experiment, the same windows were used on recorded sweeps in all experimental conditions. Stimulus artifact and fiber volley were estimated after 10 mM CNQX (6-cyano-7-nitroquinoxaline-2,3-dione) was applied at the end of most experiments, and were subtracted from each recorded field EPSP response. Experiments in which the baseline transmission drifted more than 15% (the early half vs. the late half) were excluded. At the beginning of most field EPSP experiments, we varied the stimulating intensity (input) at several levels, and measured the resulting field EPSP response (output). The stimulating intensity started at a level where no responses were observed until it reached a maximal level. Input–output lines were drawn for the linear part of the input/output curve by the standard linear regression method (Fig. 6A, below). The stimulation threshold was determined by calculating the intersection between an input–output curve and the *x*-axis (stimulation intensity). Thereafter the stimulation intensity was set midpoint between the threshold and the maximal level.

Results

INDUCTION OF LTP AT DIFFERENT AGES DURING EARLY POSTNATAL DEVELOPMENT

A number of previous studies have looked at LTP during development (Harris and Teyler 1984;

Muller et al. 1989; Dudek and Bear 1993; Bolshakov and Siegelbaum 1995). The general finding is that little or no LTP is possible at postnatal day (PND) <8. We repeated these experiments, measuring the field EPSP and inducing LTP with a strong tetanic protocol that contains five bursts (separated by 30 sec) of nine stimuli at 100 Hz (see Materials and Methods). Although we could see some persistent potentiation at young ages (Fig. 1A), the magnitude of LTP was clearly much larger at older ages (Figs. 1A–D; $n = 30$, $R = 0.49$, $P < 0.01$ at 60 min after tetanus).

We wished to determine whether transmission found in slices from younger animals lacked components necessary to express LTP, or whether there were aspects of LTP induction that were lacking. Specifically, we wished to test whether the amount of depolarization during LTP induction was insufficient in young slices. We tested this hypothesis directly by determining whether delivery of depolarizing current through a whole-cell recording electrode during LTP induction was sufficient to produce LTP in slices from young animals. We conducted a number of experiments on slices from different aged animals using whole-cell recordings in the voltage-clamp configuration (Fig. 2). After gaining whole-cell access from CA1 neurons, cells were clamped at -60 mV and synaptic transmission was elicited (See Materials and Methods). After a baseline period (5–10 min), the postsynaptic membrane was depolarized to -10 mV and synaptic transmission was delivered at 2 Hz for 75 sec. Following this induction protocol, the postsynaptic membrane was repolarized to -60 mV and synaptic transmission resumed. As shown in Figure 2A, pairing presynaptic activity with postsynaptic depolarization produced synaptic potentiation in slices from 7-day-old rats. Pooling all experiments from 5- to 9-day-old animals, we saw clear evidence for LTP (Fig. 2C). We saw no evidence that pairing-induced LTP was greater at PND 10–14 compared with PND 4–9 (no correlation at Fig. 2D, $n = 24$, $R = 0.05$, $P > 0.8$; see Fig. 5 for comparison with other types of recordings).

These results clearly show that expression of LTP is possible in slices from PND 5–9 rats. We wished to determine if the LTP observed in young animals required NMDA receptor function. We conducted the same protocol as above in the presence of $100 \mu\text{M}$ D,L-2-amino-5-phosphonovaleric acid (DL-APV), an inhibitor of NMDA receptors. Under these conditions, LTP was blocked completely, indicating that the LTP observed in slices

Figure 2: Robust LTP can be induced

in very young rats by pairing depolarization with synaptic transmission. (A) LTP in slice from a 7-day-old rat (a47a). (Top) Fifteen consecutive sweeps before (left) and after (right) pairing. (Middle) EPSC amplitude plotted vs. time. Delivery of a pairing protocol for LTP induction is indicated (see Materials and Methods). Large solid circles denote 10-point averages. Broken line denotes the average amplitude of baseline transmission. (Bottom) Series resistance plotted vs. time. (B) Similar plots as A from another experiment with a 14-day-old rat. Note the amount of potentiation in 7- and 14-day-old animals is similar. (C) Statistical analysis of 23 whole-cell LTP experiments (5–9 days old). For each experiment, the average of responses in the first half of baseline period (base1) is assigned a value of 1. There is no significant difference between the first (base1) and second (base2) half of baseline, indicating stability of transmission. Synaptic transmission is significantly potentiated at 3 min ($n = 23$; average age = 6.9 days old), 15 min ($n = 17$; 7.0 days old), 30 min ($n = 12$; 7.3-days old), and 60 min ($n = 5$; 7.2 days old) after a pairing protocol. (**) $P < 0.01$; (*) $P < 0.05$; paired t -test. (D) Synaptic potentiation at 30 min after pairing plotted vs. animal age in 24 experiments. There is no significant correlation.

from PND 6–8 behaves like conventional LTP from older animals and depends on NMDA receptor function (Fig. 3). We further wished to determine if the ability to get LTP in young animals was attributable to the depolarization provided during induction, rather than some peculiarity of whole-cell recording (wash-out of inhibitory substance, etc.). We therefore conducted a series of experiments with whole-cell recordings in current-clamp configuration. In these experiments, LTP was induced by delivering 10 bursts (separated by 3 sec) of 9 tetanic stimuli at 100 Hz after a baseline period (5–10 min). No depolarization was provided by the recording electrode. Under these conditions, LTP was significantly greater in slices from older animals, with little LTP in slices from young animals (Fig. 4, positive correlation at E; $n = 20$, $R = 0.54$, $P < 0.02$; see Fig. 5 for comparison with other types of recordings).

POSSIBLE MECHANISMS PRODUCING DEPOLARIZING DRIVE DURING TETANIC STIMULUS

The results shown indicate that LTP is possible in younger animals if sufficient depolarization

is provided during its induction. Is there less depolarization during synaptic transmission and tetanic stimulation in slices from young animals? We failed to find an age-related difference in a number of parameters examined. The initial absolute amplitudes of transmission as measured with field or current-clamp recordings could not account for the difference in LTP at different ages. Although there was a tendency for responses from younger animals to be smaller in the baseline period for field recordings ($n = 28$, $R = 0.3$, $P = 0.11$), the amplitude of the initial response did not correlate significantly with the magnitude of potentiation ($n = 28$, $R = 0.18$, $P = 0.37$). Furthermore, in current-clamp recordings there was no positive correlation between initial amplitude and age ($n = 24$, $R = -0.3$, $P = 0.13$). Additionally, we have measured synaptic facilitation by measuring the ratio of the peak amplitude during a tetanus to the amplitude of baseline transmission. We found no significant correlation between synaptic facilitation and animal age ($n = 28$, $R = 0.3$, $P > 0.1$).

Other measures, however, suggest that some aspects of depolarization may be different at different ages. Using field recordings, we constructed input–output curves (Fig. 6) and saw clear differ-

LTP IN HIPPOCAMPUS OF VERY YOUNG RATS

Figure 3: LTP in slices from young rats is blocked by APV. (A) Twelve consecutive sweeps before (left) and after (right) pairing in 100 μ M DL-APV (a49t, 6 days old). (B) EPSC amplitude plotted vs. time from the same experiment as in A. (C) Series resistance plotted vs. time from the same experiment as A and B. (D) Summary analysis of eight experiments (6–8 days old). Baseline normalization as in Fig. 2. Synaptic transmission was not significantly changed at 3, 15, or 30 min after pairing (for all intervals, $P > 0.05$, paired t -test).

ences in these curves in rats from different ages. The slope of the I/O curve as well as the threshold stimulus for obtaining a response were positively and negatively correlated, respectively, with age (Fig. 6; see also Harris and Teyler 1984). This suggests that recruitment of synaptic drive, as assessed by an extracellular stimulus, is less in younger animals.

One possible mechanism producing smaller local depolarizations is if a large fraction of synapses contain no functional AMPA receptors. Recently, we have described transmission mediated by pure NMDA receptor function in hippocampal neurons (Liao et al. 1995). This can be demonstrated clearly by reducing the stimulus intensity so that few axons are stimulated. In some cases, one can observe all failures at hyperpolarized po-

Figure 4: LTP recorded in current-clamp is age-dependent. (A) Whole-cell recording from a 6-day-old rat (a57c). (Top) Twenty consecutive individual sweeps before (left) and after (right) tetanus, with a representative response to a train of nine tetanic stimuli (middle). Only one action potential is generated during this train. (Bottom) Whole-cell EPSP amplitude plotted vs. time. Delivery of tetanus is indicated. (B) Similar plots as A in another experiment from a 12-day-old rat (a57n). Note that more spikes were evoked during a tetanic train than in A. (C) Total number of action potentials evoked during tetanus protocol plotted vs. animal age. More spikes are evoked in more mature rats ($n = 25$, $R = 0.4$, $P < 0.05$). (D) Potentiation of current-clamp EPSP amplitudes at 30 min post-tetanus plotted vs. number of spikes during tetanus protocol. Synaptic potentiation is correlated positively with number of spikes ($n = 19$, $R = 0.48$, $P < 0.04$). (E) Potentiation of current-clamp EPSP amplitudes at 30 min post-tetanus plotted vs. animal age. Synaptic potentiation is correlated positively with animal age ($n = 20$, $R = 0.54$, $P < 0.02$).

tentials with clear outward synaptic current at depolarized potentials. This can be blocked completely by APV, indicating pure NMDA receptor mediated transmission (Fig. 7A). From such sub-minimal transmission, LTP can be elicited if depolarization is provided by the recording electrode during LTP induction (Fig. 7B–D). The fraction of transmission mediated by pure NMDA receptor function can be quantified by comparing the failure rates at depolarized and hyperpolarized poten-

Figure 5: Comparison of synaptic potentiation obtained under different types of recordings for 4- to 9-day-old rats (open bars) and 10- to 14-day-old rats (solid bars). (A) Synaptic potentiation at 15 min after a LTP-inducing protocol (see Materials and Methods), respectively, in field EPSP (left), whole-cell current-clamp (middle) and whole-cell voltage-clamp recordings (right). Note significant difference in the amount of synaptic potentiation between 4- to 9-day-old rats and 10- to 14-day-old rats in both field EPSP and whole-cell current-clamp recordings ($P < 0.001$ and $P < 0.05$, respectively). However, no significant difference is found between these two groups of rats in voltage-clamp recordings ($P > 0.9$). (B) A similar plot as A at 30 min after a LTP-inducing protocol.

tials (Liao et al. 1995). This provides an estimate of the quantal content at synapses with only NMDA receptor function, relative to the quantal content at synapses with AMPA receptor function. We characterized this property in slices from different aged animals. Transmission was elicited by a weak stimulus whose intensity was set so that ~50–90% of responses were failures at hyperpolarized potentials. The failure rate was quantified by estimating the density distribution of EPSC peak amplitude and noise (see Materials and Methods). In agreement with our previous studies (Liao et al. 1995), we saw more failures when the postsynaptic membrane was held at a hyperpolarized potential than at a depolarized potential, indicating the existence of pure NMDA transmission (Fig. 8A–C). Although there was some scatter in the data, there was a clear decrease in the estimated fraction of transmission mediated purely by NMDA receptors

Figure 6: Field EPSP in the hippocampus of a younger rat has a lower input–output slope and a higher stimulation threshold. (A) Field EPSP amplitude plotted vs. stimulation intensity in three representative experiments (a47w: 14-day-old; a47s: 10-day-old; a48f: 6-day-old). (B) Input–output slope plotted vs. animal age shows positive correlation ($n = 23$, $P < 0.001$). (C) Stimulation threshold (see Materials and Methods) plotted vs. animal age shows positive correlation ($n = 23$, $P < 0.05$).

during development from PND 4 to PND 14 (Fig. 8D; $n = 34$, $R = -0.52$, $P < 0.005$). The corollary to this finding is that the fraction of release at synapses with AMPA receptor function is low at early ages, increasing from PND 4 to PND 14. This suggests the possibility that a local synaptic region may lack sufficient functional AMPA receptors required for effective postsynaptic depolarization to induce LTP.

In addition to the comparison of quantal contents of these two receptor types, we measured the ratio of net synaptic AMPA current to net synaptic NMDA current (Fig. 9). This measure will take into account not only the quantal content but also the quantal size of these two components. Interestingly, whereas the quantal components showed developmental changes, the ratio of net synaptic currents mediated by the two receptor types showed no significant changes (Fig. 9C; $n = 50$; $R = -0.04$; $P > 0.8$). This was true for the

Figure 7: Pure NMDA-receptor mediated transmission and LTP at silent synapses. (A) Average of 50 consecutive responses to a subminimal stimulus (see Materials and Methods) with holding potential -65 mV (left), $+55$ mV (middle), and $+55$ mV after application of 100 μ M DL-APV (right) in an individual experiment (a51b; 6-day-old rat). (B) Eight consecutive responses to a subminimal stimulus before (top) and after (bottom) delivery of a pairing protocol (see text); holding potential is -65 mV (a30e). (C) EPSC amplitude distributions before (top) and after pairing (bottom) in the same experiment as B. The noise distribution (thick broken line) fits the EPSC amplitude distribution before pairing, indicating the absence of responses. (D) EPSC amplitude plotted vs. time for the experiment in B and C; pairing protocol delivered were indicated.

currents obtained with minimal stimulation to estimate failure rates ($n = 35$; $R = -0.11$; $P > 0.5$) as well as for currents generated with stronger stimulus ($n = 15$, $R = -0.12$; $P > 0.7$). These results suggest the observed age-dependent differences in LTP are not attributable to a larger NMDA current in more mature rats.

Are there other measures indicating inadequate depolarization during LTP induction in slices from young animals? There has been interest recently in the role of action potential generation in relation to synaptic integration and plasticity (Jaffe et al. 1992; Spruston et al. 1995; Magee and Johnston 1995). With whole-cell current-clamp recordings, we examined the number of spikes generated during a tetanus for different age animals (Fig. 4A,B, insets). Plotting the total number

of spikes generated during the tetanic protocol delivered to induce LTP versus age of animal, we found a positive correlation (Fig. 4C; $n = 25$; $R = 0.4$; $P < 0.05$). This indicates that fewer action potentials are generated during tetanic stimulation in neurons from slices of younger animals. Furthermore, there was a significant positive correlation between the number of spikes generated during LTP induction and the magnitude of potentiation at 5, 15, and 30 min after a tetanus (5 min: $n = 22$, $P < 0.001$; 15 min: $n = 21$, $P < 0.04$; 30 min: $n = 19$, $P < 0.04$; Fig. 4D).

Discussion

In this study we have attempted to answer one question: Why is there less LTP in tissue from 1-week-old rats compared with 2-week-old rats? In agreement with previous studies, we find that the magnitude of LTP as measured in field or current-clamp recordings increases between PND 4 and 14 (Figs. 1 and 4). Although LTP at 1 week is smaller than at 2 weeks, there is nevertheless clear LTP in young animals with field recordings (e.g., Fig. 1A). Several proposals have been made to explain this developmental progression. One possibility is that the biochemical machinery involved in modifying synapses is developmentally regulated and is lacking in 1-week-old animals. Another possibility is that synapses initially have a saturated amount of LTP and that during development synapses get weaker and therefore allow LTP to be expressed (Bolshakov and Siegelbaum 1995).

To address this issue we have considered the steps hypothesized to occur in the generation of LTP. A tetanic stimulus recruits activity in presynaptic axons in a repetitive manner. Transmitter is released producing spatio-temporal summation of EPSPs and action potentials. This depolarization allows opening of postsynaptic NMDA receptors and entry of calcium. These events are considered transient and therefore part of the steps involved in LTP induction. These events are thought to cause the biochemical machinery of synapses to be modified in such a manner that transmission is enhanced permanently. The cellular mechanisms that keeps the biochemical machinery in this modified state are considered to be responsible for the maintenance of LTP. The specific effect of this modified machinery is considered to be the site of LTP expression. We have asked if the small amount of LTP in young tissue is attributable to a defi-

ciency in the induction, maintenance, or expression of LTP.

Our data argues for a defect in the induction and not the maintenance or expression of LTP. Specifically, our results support the view that in young tissue, a tetanic stimulus does not provide the necessary depolarization to induce LTP. The inability to generate LTP in young slices is not simply because we produced smaller baseline responses in slices from young animals. There appears to be a more subtle aspect of depolarization during tetanic stimulus, rather than the amplitude of baseline transmission, that determines potentiation. It is possible that the amount of synaptic drive in any local dendritic region could be lower in younger animals. This is one common way to interpret three different findings concerning basal transmission in younger animals: the shallower I/O slope, the higher stimulus threshold necessary to produce a response, and the higher fraction of transmission mediated by pure NMDA receptors. If a large local depolarization is necessary to open synaptic NMDA receptors, then this interpretation would predict less NMDA receptor activation during tetanic stimulus and less LTP. Furthermore, we see fewer action potentials generated during a tetanus in young animals and a significant correlation between the number of action potentials generated and the amount of LTP. These data provide evidence that the amount and nature of depolar-

ization provided by tetanic stimulation is significantly different in younger animals and this difference contributes to the smaller amount of LTP found in these animals.

The strongest support of the hypothesis that an insufficient depolarization during LTP induction is responsible for the diminished LTP in young animals is the finding that we can get robust LTP in slices from young animals if depolarizing current is provided during LTP induction (Figs. 2 and 5). This finding clearly indicates that the biochemical machinery sufficient for expression and maintenance of LTP is present in 1-week-old animals. This finding also counters directly a recently proposed hypothesis regarding the development of LTP. It was proposed (Bolshakov and Siegelbaum 1995) that the diminished LTP in young animals was attributable to a saturation of expression mechanisms. Specifically, it was proposed that the probability of transmitter release (Pr) is close to 1 (i.e., saturated) in young animals and that LTP is caused by an increase in Pr . Furthermore, it was proposed that as the animal matures there is a decrease in Pr , and thereby allowing for the expression of LTP by increases in Pr . However, we clearly see LTP with both field (Fig. 1) and whole-cell recordings (Figs. 2 and 4) that is blocked by APV (Fig. 3) at ages where it was claimed that Pr is 1 and LTP was saturated. This finding is not consistent with the claims that: (1)

Figure 9: The ratio of synaptic AMPA current/NMDA current is not correlated with animal age. (A) Averaged EPSCs at depolarized (top, +55 mV) and hyperpolarized (bottom, -65 mV) potential. Broken lines represent windows selected for estimating the base line, AMPA current (at hyperpolarized potential) and NMDA current (at depolarized potential) (a42e, 6 days old). (B) Records similar to A from a 14-day-old rat (a43a). (C) The ratio of AMPA current/NMDA current plotted vs. animal age. No correlation is found between the ratio and animal age ($n = 50$, $R = -0.04$, $P > 0.8$).

$Pr = 1$ in young animals and (2) LTP is attributable to an increase in Pr . At least one of these two statements is inconsistent with our results. Alternative explanations for the observations of the Boshakov and Siegelbaum study (1995) are that (1) their experimental conditions biased toward selection of a small subgroup of synapses that were not representative of the whole population of synapses that we see with stronger stimulation or (2) their experimental conditions biased against the induction (rather than expression) of LTP.

As in a previous study, we have estimated the fraction of synaptic events acting purely on NMDA receptors by comparing the failure rate at depolarized and hyperpolarized potentials (Liao et al. 1995). With this estimate, we find that there is a higher prevalence of release at pure NMDA synapses earlier in development. At PND 4–5 almost

80–90% of release appears to occur at synapses that transmit with only NMDA receptors. By PND 13–14 this fraction is ~50%. A recent study (Durand et al. 1996) also reached a similar conclusion regarding the age-dependent prevalence of pure NMDA-mediated transmission using a different stimulation protocol. However, unlike our estimate, their estimate of pure NMDA receptor mediated transmission depends on the assumption that (1) they can reliably stimulate a single pre-synaptic fiber and (2) that a single fiber makes a single contact. These assumptions are not universally accepted.

The decrease in pure NMDA-receptor mediated transmission during development parallels what we have described previously to occur during LTP (Liao et al. 1995). This observation supports the possibility that an LTP-like process occurs during development, converting pure NMDA receptor synapses to synapses with mixed AMPA and NMDA receptor function. Pairing-induced LTP cannot account for all synaptic changes observed during development, however. For instance, pairing-induced LTP produces an increase in the ratio of synaptic current mediated by AMPA receptors relative to NMDA receptors (Liao et al. 1995). This differs from what we find during development of these neurons, where no significant change is detected in the AMPA/NMDA ratio in synaptic currents. It is notable that more comparable changes in AMPA and NMDA receptor-mediated currents can occur during tetanus-induced LTP (Tsien and Malinow 1990; Bashir et al. 1991; O'Connor et al. 1995). How do we interpret the lack of change in AMPA/NMDA ratio along with the change in the fraction of transmission occurring at pure NMDA synapses? The latter is a measure of the relative quantal contents of synapses using these different receptors, whereas the former is a measure of the quantal content and quantal size. These observations can be reconciled if a change in quantal size of the AMPA and NMDA synapses offsets the change in quantal contents. This could be if quantal size of synapses with AMPA receptors decrease during development or if the quantal size of NMDA increases with development.

In conclusion, we find that in slices from young animals LTP can be induced if sufficient depolarization is provided during its induction. We also find that during tetanically induced LTP there are some aspects of depolarization that are diminished in slices from young animals. These along with other factors under developmental

control are likely to limit the induction of LTP in young animals. It remains to be seen whether there are mechanisms used in vivo that obviate this limitation.

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